

In the specification:

Please replace the 2<sup>nd</sup> complete paragraph on page 6 with the following:

Figs. 1A through 1D show shows four expression cassettes used to generate transgenic mice. (A) -179 lacZ $\epsilon$  $\mu$ LCR (MB70) is the basic construct with the minimal  $\epsilon$ -globin promoter ( $\epsilon$ -pro), extending to -179 with respect to the start site of transcription (+1) and ~20 bp of  $\epsilon$ -globin 5'-untranslated region (shown as small black box below +1).  $\epsilon$ -pro is linked to a LacZ expression cassette containing a Kozak consensus sequence and translational start site (SDK region). Downstream from the LacZ reporter gene is a portion of the 3'-region of the  $\epsilon$ -globin gene containing part of exon 2, all of intron 2 (IVS 2) and all of exon 3; these sequences are shown as black boxes (exons) and a black line (intron). The 3'-untranslated region (containing the polyadenylation site, pA) is shown as a striped line. A truncated version of the LCR (the  $\mu$ LCR) is located downstream from the  $\epsilon$ -lacZ sequences. (B) -849 lacZ $\epsilon$  $\mu$ LCR (MB73), (C)  $\epsilon$ -PRE(II+V)lacZ $\epsilon$  $\mu$ LCR (MB72); and (D) -2kblacZ $\epsilon$  $\mu$ LCR (MB92). A-D contain  $\epsilon$ -pro and different portions of the upstream regulatory region of the human  $\epsilon$ -globin gene. Eukaryotic sequences in A-D were excised from the vector by digestion with KpnI and NotI and then purified for microinjection into the male pronuclei of mouse zygotes.

Please replace the 3<sup>rd</sup> complete paragraph on page 6 with the following:

Figs. 2A through 2F show shows LacZ expression, correlated with the appearance of a blue stain, in primitive erythroblasts. (A) (a) is a diagrammatic representation of a 7.5 dpc embryo; (Bb) is a transgenic embryo stained with XGal and viewed by bright field microscopy, (Ce) is the same embryo viewed by dark field microscopy; B (a) (D-E) is a non-transgenic mouse; (b) and (c) are embryos at 8.5 dpc stained with XGal; E(a) (F) is a wild type 12.5 dpc embryo; (b) and (c) are transgenic 12.5 dpc embryos. (1) wild type; (2) transgenic; (3) ectoplacental cone; (4) blood islands; (5) amniotic cavity; (6) trophectoderm; (7) allantois; (8) extraembryonic mesoderm; (9) embryo proper (epiblast).

Please replace the 1<sup>st</sup> complete paragraph on page 7 with the following:

Figs. 3A through 3D show shows the formation of yolk sac-like structures by cultured blastocysts: (A) (a) transgenic blastocysts prior to culture; (B) (b) Saesac-like structure (non transgenic) stained with benzidine to reveal hemoglobin containing cells; (C) (c) Sae sac from cultured transgenic blastocysts stained with XGal to reveal hemoglobin containing cells after 9 days of cultivation; and (D) (d) Normal normal 8.5 dpc transgenic embryo and yolk sac stained with XGal.

Please replace the 5<sup>th</sup> complete paragraph on page 7 with the following:

Figs. 7-1A through 7-1D show shows that when transgenic explants of gastrulating embryos (isolated at 6.25-6.5dpc) are cultured on filters or glass slides for 72 hours, induction of embryonic hematopoiesis occurs in whole embryo, but is absent in epiblasts only, as determined by XGal staining. Dashed lines were drawn around the epiblasts to facilitate visualization of structures. 7A1 (7-1A) whole embryo on a filter; 7A2 (7-1B) epiblast on a filter; 7A3 (7-1C) whole embryo on a slide; and 7A4 (7-1D) epiblast on a slide.

Please replace the 1<sup>st</sup> complete paragraph on page 8 with the following:

Figs. 7-2A through 7-2E show 7-1 shows blood formation in transgenic embryonic explant cultures. (lacZ stained sections of embryos). Frozen tissue sections were XGAL stained to reveal cluster of lacZ-positive hematopoietic cells in the whole embryos (7-2A) 7B1, epiblasts (7-2B) 7B2, posterior embryo portions (7-2C, 7-2D), 7B3, 7B4 and transgenic anterior epiblast portion adjacent to the VE (7-2E), 7B5, but not in surrounding visceral endoderm and undifferentiated mesoderm nor in the nontransgenic VE tissue of anterior/VE recombinants. (e)

Please replace the 2<sup>nd</sup> complete paragraph on page 8 with the following:

Figs. 8-1A through 8-1C show shows induction of hematopoiesis by visceral endoderm (VE) signals. A (8-1A) dark-field photomicrograph of recombinant containing transgenic (Tg) epiblast and non-Tg VE showing localized lac Z staining in the embryo adjacent to the visceral endoderm; B (8-1B) schematic diagram corresponding to panel

1Aa. Abbreviations: Tg, transgenic; Ve, visceral endoderm; EryP, primitive erythroid cells;  $\in$  (8-1C) bright field photomicrograph of recombinant shown in A 1A.

Please replace the 3<sup>rd</sup> complete paragraph on page 8 with the following:

Fig. 8-2 8D shows induction of embryonic hematopoiesis in whole embryo, and in epiblast plus visceral endoderm, but none in epiblasts only, using RT-PCR. (All samples were prepared following a 72 hour *in vitro* incubation of embryos isolated at 6.5 dpc). Actin served as an internal control.

Please replace the 1<sup>st</sup> complete paragraph on page 9 with the following:

Figs. 14A through 14F show shows the early development of the mouse at 2.5 dpc (A), 3.5 dpc (B), 4.5dpc (C), 5.5 dpc (D), 6.5 dpc (E), and 7.5 dpc (F). The region of early blood island formation occurs in the exocoelomic cavity (see F f) between the epiblast below which is surrounded by the visceral endoderm and the extraembryonic tissue above.

Please replace the 2<sup>nd</sup> complete paragraph on page 9 with the following:

Figs. 15A through 15C show shows the experimental scheme for separation of epiblast into anterior and posterior portions. (A) depicts the entire 6.75 dpc embryo with visceral endoderm around the perimeter of the epiblast and the extraembryonic mesoderm. (B) depicts the embryo after the visceral endoderm has been stripped off and (C) shows the epiblast only, with a dotted line of transection showing how the anterior and posterior sections are physically divided before separate cultivation.

Please replace the 3<sup>rd</sup> complete paragraph on page 9 with the following:

Figs. 16-1 shows that hematopoietic mesoderm arises from the posterior primitive streak (posterior mesoderm) when anterior and posterior portions of lacZ transgenic embryos are harvested at mid- to late-gastrulation. Top Panel-A: No staining is detected in anterior epiblasts. Bottom Panel-B: Dark blue XGAL histochemical staining shows blood formation in cultured posterior epiblasts. Scale bar, 1mm.

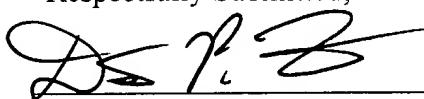
## CONCLUSION

In view of the foregoing amendments to the specification and Applicants' submission of formal drawings, Applicants submit that the pending application is in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945**.

Respectfully Submitted,

Date: August 28, 2003

**Customer No: 28120**  
Docketing Specialist  
Ropes & Gray  
One International Place  
Boston, MA 02110  
Phone: 617-951-7000  
Fax: 617-951-7050



---

David P. Halstead, Ph.D.  
Reg. No. 44,735